Regulation of Sugar Transport via the Multiple Sugar Metabolism Operon of *Streptococcus mutans* by the Phosphoenolpyruvate Phosphotransferase System

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In this report, we provide evidence that the transport of sugars in *Streptococcus mutans* via the multiple sugar metabolism system is regulated by the phosphoenolpyruvate phosphotransferase system. A *ptsI*-defective mutant (DC10), when grown on the multiple sugar metabolism system substrate raffinose, exhibited reduced growth, transport, and glycolytic activity with raffinose relative to the parent strain BM71. Inhibition of [^{3}H]raffinose uptake was also observed in both BM71 and DC10 with increasing concentrations of glucose and the glucose analogs α-methyl glucoside and 2-deoxyglucose.

Streptococcus mutans is capable of generating acid end products during carbohydrate metabolism, which contributes to the demineralization of tooth enamel, leading to dental caries (9). The primary sugar transport system utilized by *S. mutans* is the phosphoenolpyruvate:sugar phosphotransferase system (PTS) (6, 8, 17). The PTS regulates the transport and metabolism of non-PTS sugars in both gram-positive and gram-negative bacteria either by direct phosphorylation of target proteins of non-PTS systems or by protein-protein interactions of target proteins of the PTS and of non-PTS systems with components of the PTS (10, 12). In order to study non-PTS glucose transport, we constructed by allelic exchange a mutant of *S. mutans* BM71 defective in *ptsI*, the gene for the general PTS protein enzyme I (EI) (2). This mutant, designated DC10, was unable to phosphorylate EI and HPr with [^{32}P]phosphoenolpyruvate and would not grow or transport PTS sugars, except glucose, indicating the presence of a non-PTS glucose transport system in *S. mutans* (2).

The multiple sugar metabolism transport system (MSM) in *S. mutans* is a non-PTS sugar uptake system involved in the transport and metabolism of melibiose, raffinose, and isomaltosaccharides (14). We have previously demonstrated diauxic growth of *S. mutans* BM71 on a mixture of glucose and melibiose with the utilization of melibiose inhibited by glucose, suggesting PTS-mediated regulation of the MSM (1). In the present study, we examined the growth, sugar transport, and glycolytic rates of BM71 and the *ptsI* mutant DC10 with glucose and raffinose as substrates. We also report the effects of glucose and glucose analogs on [^{3}H]raffinose transport by BM71 and DC10, which demonstrate a direct effect of these PTS substrates on transport via the MSM.

Since early experiments (2) characterizing the *ptsI* mutant DC10 had demonstrated that the mutant was able to utilize the MSM substrates raffinose and melibiose, it was anticipated that growth with raffinose would be similar to that of the parental organism, because the MSM and the PTS were believed to be independent transport systems. In order to test this prediction, we first examined the anaerobic growth of BM71 and DC10 with 0.3% glucose or raffinose in tryptone-yeast extract (TYE) broth, with growth monitored in a Klett-Summerson colorimeter with a red filter. As seen in Fig. 1, the mutant grew more

slowly on glucose than BM71, as expected (2); however, growth on raffinose was also significantly slower than that of the parental strain. Calculation of the doubling times revealed that those for DC10 were four- and twofold higher with glucose and raffinose, respectively, than those for BM71 (Table 1). Sugar transport rates, measured by using 1 mM [^{14}C]glucose (9 μCi μmol^{-1}) or 250 μM [^{3}H]raffinose (450 μCi μmol^{-1}) (7), revealed that transport activities of intact, washed cells of DC10 were only 1 and 13% of those of BM71, respectively (Table 1). In addition, the glycolytic rate of the mutant with raffinose as the carbon source, as measured by the rate of acid production in a pH-stat at pH 7.0 (7), was only 7% of that of BM71 (Table 1). These substantial differences between the wild-type strain and the mutant with respect to growth, transport, and glycolytic activity with raffinose as the substrate indicated an impairment of MSM transport and metabolism, suggesting that the PTS was involved in the regulation of the MSM.

In gram-negative bacteria (15), and in certain cases in gram-positive bacteria (3, 4, 11, 16), tight *ptsI* mutations (such as that in DC10) will yield a phenotype showing no, or limited, ability
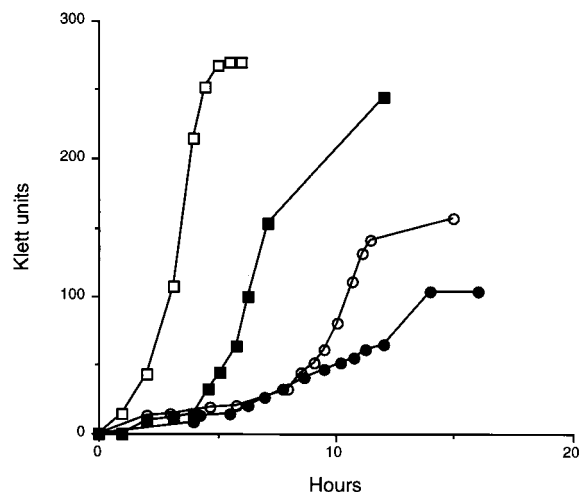

FIG. 1. Growth of wild-type *S. mutans* BM71 and the *ptsI* mutant DC10 on glucose and raffinose. □, BM71-glucose; ■, BM71-raffinose; ●, DC10-glucose; ○, DC10-raffinose. Cells were grown in TYE broth with 0.3% sugar.

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TABLE 1. Growth, sugar transport, and glycolytic rates generated by washed cells of wild-type *S. mutans* BM71 and the *ptsI* mutant DC10[a]

Strain	Growth rate with[b]:		Sugar transport rate[c]		Glycolytic rate with[d]:	
	Glucose	Raffinose	Glucose	Raffinose	Glucose	Raffinose
BM71	50 ± 5	76 ± 4	115 ± 12	9 ± 1.5	418 ± 33	475 ± 33
DC10	199 ± 5	150 ± 4	1.4 ± 0.3	1.2 ± 0.1	56 ± 8	35 ± 5

[a] Data are means ± standard deviations.
[b] Growth measured as the doubling time (in minutes) in TYE medium with 0.3% sugar.
[c] Nanomoles of sugar transported $\cdot$ mg of dry cells$^{-1} \cdot$ min^{-1}.
[d] Nanomoles of acid neutralized $\cdot$ mg of dry cells$^{-1} \cdot$ min^{-1} at pH 7.0.

to utilize non-PTS substrates for growth. Although we did not observe a complete loss of raffinose transport activity, the reduced ability of the PTS-defective mutant to metabolize the MSM substrates suggested control of MSM transport by the PTS. The decreased MSM transport observed with *S. mutans* DC10 was similar to the decreased ability of a *ptsI* mutant of *Enterococcus faecalis* to utilize glycerol (3). Recent work with *Streptococcus salivarius* suggests that *ptsI* mutants of this organism also behave differently from enteric bacteria, since tight mutations produced a phenotype able to utilize lactose, a non-PTS substrate for this organism (5).

Since the PTS is the primary sugar transport system in *S. mutans* (6), it was hypothesized that an increase in PTS transport would likely lead to inhibition of uptake via any non-PTS secondary system, including the MSM. To study the effects of PTS substrates on [³H]raffinose transport, unlabelled glucose was added at concentrations between 0.1 and 1 mM to reaction mixtures containing labelled raffinose and washed raffinose-
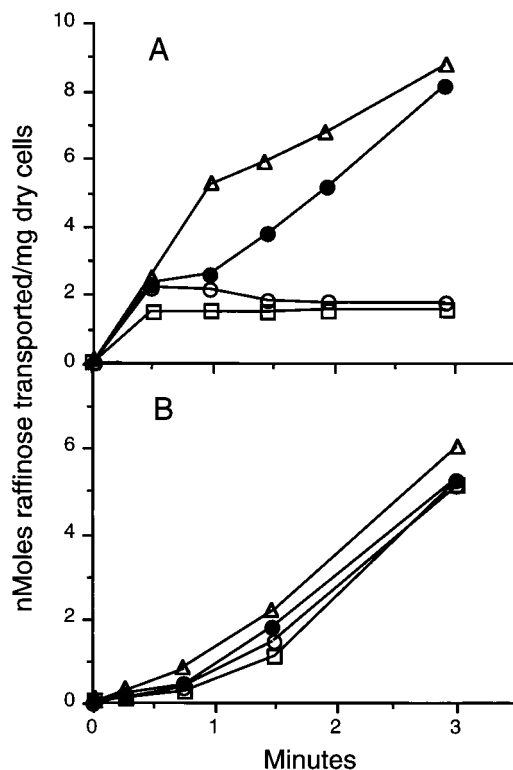

FIG. 2. Effects of glucose on the transport of [³H]raffinose in raffinose-grown cells of *S. mutans* BM71 (A) and the *ptsI* mutant DC10 (B). The concentrations of glucose added to the reaction mixtures were as follows: △, 0 mM (control); ●, 0.1 mM; ○, 0.5 mM; and □, 1.0 mM.

grown cells of BM71 or the mutant DC10. As seen in Fig. 2, BM71 typically demonstrated a three- to fivefold-higher rate of raffinose transport than did the *ptsI* mutant and glucose concentrations as low as 0.1 mM had a pronounced inhibitory effect on raffinose uptake by BM71. Conversely, glucose had only a small inhibitory effect on raffinose transport by the mutant at glucose concentrations as high as 1 mM. Similar effects were observed with the glucose analogs α-methylglucoside and 2-deoxyglucose (data not shown). The inhibitory effects of these PTS substrates on raffinose uptake by mutant DC10 were not as severe as those observed with most *ptsI* mutants of enteric bacteria (15). In these bacteria, enzyme I is believed to perform a catalytic role in relieving inhibition of non-PTS transport by a PTS-mediated mechanism by decreasing the ratio of P-IIA^{Glc} to IIA^{Glc}, which in turn modulates the activity of non-PTS transport.

PTS-mediated regulation of non-PTS systems in gram-positive bacteria has been shown to involve phosphorylation of non-PTS target proteins. This phenomenon has been demonstrated to occur in the regulation of glycerol utilization in *E. faecalis* (4, 13). Preliminary experiments utilizing [³²P]phosphoenolpyruvate in reactions with cell extracts of both melibiose- and glucose-grown BM71 indicated the presence of a 59-kDa phosphoprotein that appears to be specific to MSM substrate-grown cells since it was not detected in extracts of glucose-grown BM71 (data not shown). The phosphorylation of this 59-kDa protein was also dependent on a functional PTS, since no detectable phosphoproteins were observed in extracts prepared from raffinose- or melibiose-grown cells of the *ptsI* mutant DC10.

The physiological experiments described lend strong support to the concept that *S. mutans* preferentially utilizes the PTS over the MSM in the transport of sugars. These results and previous data (1) suggest that the PTS modulates both the expression and the transport activity of the MSM. The mechanism of signal transduction has not been elucidated, but it may involve phosphorylation of the 59-kDa phosphoprotein.

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